

RISZU2'-3 (4 CODONS)

SEQ ID NO:47 5'

GT GAC GCC CCT GTA CT 3'

RISZU2'-4 (3 CODONS)

SEQ ID NO:48 5'

GT GAC GCC CCT GT 3'

RESULTS AND CONCLUSIONS:

31' mcd  
As described in Methods, primer 2' derivatives vary in length from 15-18 bp that could encode a peptide of 4-5 amino acids in length. Figure 3 shows that PCR-amplified products were generated using primer 1' and primer 2' derivatives 1, 2, and 3 and all three genomic DNAs as a source of target polynucleotides.

These results demonstrate that the method as described can utilize conserved regions of greater than or equal to 4 amino acids in length for use in isolating/identifying gene orthologs from different plant families.--

In the Claims:

Please cancel claims 32 and 33.

Please amend the claims as follows.

abc  
B2  
1. (Amended) A method for isolating from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as corresponding nucleotides in nucleotides in the template polynucleotide;

B2  
cont  
(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of a most preferred codon of the target plant class for a desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex comprising the target polynucleotide.

wbc2  
23  
4. (Amended) The method of claim 1, wherein wherein the sequence or the oligonucleotide of step (b) or its reverse complement further comprises at least one codon wherein

(i) the sequence of the first and second position of the codon is the same as corresponding nucleotides in the template polynucleotide;

(ii) the sequence of the third position of the codon of step (I) is the same as the nucleotide of the third position of a second most preferred codon of the target plant species for a desired amino acid; and

(iii) the oligonucleotide is not degenerate.

sub C3  
B4 7. (Amended) The method of claim 5, wherein the third position of each codon of the oligonucleotide is either a guanosine or cytosine.

sub C4  
B2 10. (Amended) The method of claim 9, wherein the third position of each codon of the oligonucleotide is either an adenosine or thymidine.

sub C5  
B6 15. (Amended) The method of claim 1, wherein step (a) comprises aligning the sequences of polynucleotides of plants within a family and identifying a portion of the template polynucleotide that exhibits at least 70% sequence identity to a portion of a polynucleotide from a plant of a genus closely related to the plant from which the template polynucleotide originates.

sub C6  
B7 22. (Amended) A method of isolating from a target organism a target polynucleotide encoding a conserved region in a template polypeptide encoded by a template polynucleotide comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the conserved region of step (a), wherein

(i) the sequence of the first and second positions of at least three codons is the same as corresponding nucleotides in the template polynucleotide;

ubc6  
B7  
CONF  
(ii) the nucleotide of the third position of the at least three codons of the oligonucleotide is the same nucleotide in the third position of the most preferred codon of the target organism for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex;

(d) contacting the duplex of step (c) with a thermostable polymerase under conditions to elongate the oligonucleotide of step (b); and

(e) isolating the elongation product of step (d) as the target polynucleotide.

23. (Amended) A method for identifying in a target organism a target polynucleotide encoding a conserved region in a template polypeptide encoded by a template polynucleotide comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

BC6  
(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a portion of the conserved region of step (a), wherein

27  
cont  
(i) the sequence of the first and second positions of at least three codons is the same as corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of the at least three codons of the oligonucleotide is the same nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex;

(d) contacting the duplex of step (c) with a thermostable polymerase under conditions to elongate the oligonucleotide of step (b); and

(e) determining the nucleotide sequence of the elongation product of step (d), thereby identifying the target polynucleotide.

24. (Amended) A method of isolating from a target plant species a target polynucleotide encoding a polypeptide of a conserved region in a template polypeptide encoded by a template polynucleotide, comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating a first oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a first portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of the codons of step (i) is the same as the nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) generating a second oligonucleotide wherein its sequence or its reverse complement comprises four codons that encode a second portion of the conserved region of step (a), wherein

abc6  
(i) the sequence of the first and second position of at least three codons is the same as corresponding position in the template polynucleotide;

B7  
conceded  
(ii) the nucleotide of the third position of those codons is the same as the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(d) contacting the first and second oligonucleotides with a composition comprising the target polynucleotide under conditions that permit hybridization of at least one of the oligonucleotides and the target polynucleotide to form a duplex;

(e) contacting the duplex of step (d) with a thermostable polymerase under conditions to elongate the at least one hybridized oligonucleotide;

(f) generating a strand complementary to the elongation product of step (e); and

(g) isolating the product of step (f) as the target polynucleotide.

abc7  
27. (Amended) The method of claim 24, wherein the product of step (g) is inserted into a vector.

28. (Amended) A method for identifying in a target plant species a target polynucleotide encoding a polypeptide of a conserved region in a template polypeptide encoded by a template polynucleotide, comprising:

(a) identifying the amino acid sequence of the conserved region in the template polypeptide;

(b) generating a first oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises four codons that encode a first portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as corresponding nucleotides in the template polynucleotide;

(ii) the nucleotide of the third position of the codons of step (i) is the same as the nucleotide in the third position of the most preferred codon of the target plant species for the desired amino acid;

(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(c) generating a second oligonucleotide wherein its sequence or its reverse complement comprises four codons that encode a second portion of the conserved region of step (a), wherein

(i) the sequence of the first and second position of at least three codons is the same as corresponding position in the template polynucleotide;

sub 7  
(ii) the nucleotide of the third position of those codons is the same as the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

28  
cont  
(iii) the oligonucleotide does not comprise homopolymers of more than four nucleotides; and

(iv) the oligonucleotide is not degenerate;

(d) contacting the first and second oligonucleotides with a composition comprising the target polynucleotide under conditions that permit hybridization of at least one of the oligonucleotides and the target polynucleotide to form a duplex;

(e) contacting the duplex of step (d) with a thermostable polymerase under conditions to elongate the at least one hybridized oligonucleotide;

(f) generating a strand complementary to the elongation product of step (e); and

(g) determining the nucleotide sequence of the product of step (f), thereby identifying the target polynucleotide.

29. (Amended) A method for designing a nucleotide sequence for an oligonucleotide primer for a polymerase chain reaction comprising:

(a) selecting a nucleotide sequence encoding a desired amino acid sequence from a template organism, or the complement thereof;

1517  
(b) selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

28  
cont  
(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a polyguanylate or polycytidylate sequence of more than four residues;

(d) thereby obtaining a nucleotide sequence for an oligonucleotide primer;

wherein said desired amino acid sequence is encoded by one reading frame, or a portion thereof, of the nucleotide sequence of said primer or the complement thereof.

30. (Amended) A method for preparing an oligonucleotide primer for a polymerase chain reaction comprising:

i) designing a nucleotide sequence by;

(a) selecting a nucleotide sequence encoding a desired amino acid sequence from a template organism, or the complement thereof;

(b) selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

287  
(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a polyguanylate or polycytidylate sequence of more than four residues; and

28  
cont  
ii) synthesizing an oligonucleotide primer comprising the designed sequence, wherein said desired amino acid sequence is encoded by one reading frame, or a portion thereof, of the nucleotide sequence of said primer or the complement thereof.

31. (Amended) A method for cloning a nucleic acid comprising:

i) designing a pair of oligonucleotide primers by;

(a) selecting an upstream nucleotide sequence encoding a first desired amino acid sequence from a template organism and a downstream nucleotide sequence encoding a second desired amino acid sequence;

(b) for each of said upstream and downstream nucleotide sequences, selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a polyguanylate or polycytidylate sequence of more than four residues.

ii) synthesizing an upstream oligonucleotide primer and a downstream oligonucleotide primer comprising the nucleotide sequences designed according to steps (b) and (c); and

iii) performing a polymerase chain reaction using said upstream and downstream primers and a template comprising a nucleic acid sample obtained from said target organism, thereby obtaining a cloned nucleic acid.

34. (Amended) The method of claim 31, further comprising:

(f) screening a library prepared from nucleic acids obtained from said target organism using the product of said polymerase chain reaction of step (e) as a probe .

35. (Amended) The method of claim 31, further comprising:

(f') inserting the product of the polymerase chain reaction of step (e) into a vector.

36. (Amended) The method of any one of claims [30-35] 31, 34 or 35, wherein said template organism is a dicot and said target organism is a monocot or wherein said template organism is a monocot and said target organism is a dicot.

37. (Amended) A method for isolating a target polynucleotide encoding a target polypeptide comprising a conserved region of a

template polypeptide that is encoded by a template polynucleotide, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) elongating said oligonucleotide to form a single strand polynucleotide and isolating the elongation product as the target polynucleotide.

38. (Amended) A method for isolating from a target plant species a target polynucleotide encoding a target polypeptide

comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of a plant family of target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex, thereby isolating the target polynucleotide.

39. (Amended) A method for isolating from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of a genus of the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex, thereby isolating the target polynucleotide.

40. (Amended) A method for isolating from a target plant species a target polynucleotide encoding a target polypeptide comprising a conserved region exhibiting at least 70% sequence identity to a conserved region of template polypeptide that is encoded by a template polynucleotide from a template plant species, comprising:

(a) identifying an amino acid sequence of a conserved region in the template polypeptide;

(b) generating an oligonucleotide comprising a sequence wherein the sequence or its reverse complement comprises at least four codons that encode a portion of the amino acid sequence of (a), wherein

(i) the sequence of the first and second positions of at least three of the codons is the same as corresponding nucleotides in nucleotides in the template polynucleotide;

(ii) the nucleotide at the third position of the codons of (i) is the nucleotide of the third position of the most preferred codon of the target plant species for the desired amino acid;

(c) contacting the oligonucleotide with a composition comprising the target polynucleotide under conditions that permit hybridization of the oligonucleotide to the target polynucleotide to form a duplex; and

(d) isolating the duplex, thereby isolating the target polynucleotide.

Please add the following new claim:

---

--41. A method for isolating a nucleic acid comprising:

i) designing at least one oligonucleotide probe by;

(a) selecting an upstream nucleotide sequence encoding a first desired amino acid sequence from a template organism and a downstream nucleotide sequence encoding a second desired amino acid sequence;

B11 (b) for each of said upstream and downstream nucleotide sequences, selecting for the nucleotide of the third position of each codon the preferred codon for a target organism, provided said nucleotide is guanine or cytosine;

(c) if the nucleotide of the third position of the preferred codon is adenine or thymine, then substituting either a guanine or cytosine, selecting guanine or cytosine to avoid introducing a poly-guanylate or polycytidylate sequence of more than four residues.

ii) synthesizing said at least one oligonucleotide probe comprising the nucleotide sequences designed according to steps (b) and (c); and

iii) hybridizing said at least one oligonucleotide probe to a sample comprising a nucleic acid obtained from said target organism, thereby identifying a target nucleic acid; and

iv) isolating said target nucleic acid.--

---